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Extra-hepatic Morbidity and Mortality of Chronic Hepatitis C

Short title: Extra-hepatic manifestations of HCV (35 characters, including spaces; limit 45)

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Abbreviations: BMI, body-mass index; CI, confidence interval; DAA, direct-acting antiviral; HOMA-IR, homeostasis model for assessment of insulin resistance; HR, hazard ratio; HRQL, health-related quality of life; IDO, indoleamine-2-3-dioxygenase; IRS, insulin receptor substrate; JNK, c-Jun N-terminal kinase; MRS, magnetic resonance spectroscopy; mTORC1, mammalian target of rapamycin complex 1; NS3, non-structural protein 3; PET, positron emission tomography; SOCS, suppressor of cytokine signaling; SVR, sustained virologic response; TNF- α , tumor necrosis factor alpha

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All authors contributed to the planning and drafting of the manuscript, and provided critical revision of the manuscript for important intellectual content. All authors approved the final version of the manuscript for submission.

Conflicts of Interest

FN is a member of the advisory boards of MSD, Gilead, AbbVie, Janssen, and BMS; has received consulting fees from MSD, Boehringer Ingelheim, and Janssen; and has received grants from Roche and Gilead, and lecture fees from Gilead.

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Abstract

Chronic hepatitis C virus (HCV) infection is associated with several extra-hepatic manifestations. Patients with HCV may develop mixed cryoglobulinemia and its sequelae, ranging from cutaneous and visceral vasculitis to glomerulonephritis and B cell non-Hodgkin's lymphoma. HCV-infected patients have increased rates of insulin resistance, diabetes and atherosclerosis, which may lead to increased cardiovascular morbidity and mortality. Neurologic manifestations of HCV infection include fatigue and cognitive impairment. The mechanisms causing the extra-hepatic effects of HCV infection are likely multifactorial and may include endocrine effects, HCV replication in extra-hepatic cells, or a heightened immune reaction with systemic effects. Successful eradication of HCV with interferon alpha and ribavirin was shown to improve some of these extra-hepatic effects: sustained virologic response is associated with resolution of complications of cryoglobulinemia, reduced levels of insulin resistance, reduced incidence of diabetes and stroke, and improved fatigue and cognitive functioning. The availability of new interferon-free, well-tolerated anti-HCV treatment regimens is broadening the spectrum of patients available for therapy, including those in whom interferon was contraindicated, and will likely result in greater improvements in the extra-hepatic manifestations of HCV. If these regimens are shown to confer significant benefit in the metabolic, cardiovascular, or neuropsychiatric conditions associated with HCV infection, extra-hepatic manifestations of HCV may become a major indication for treatment even in the absence of liver disease.

Key words: cryoglobulins, insulin resistance, cardiovascular risk, fatigue, health-related quality of life

Introduction

Treating patients chronically infected with hepatitis C virus (HCV) to eradicate the infection and achieve sustained virologic response (SVR; undetectable HCV RNA 12 or 24 weeks after the completion of therapy) decreases the risk of cirrhosis, liver failure, and hepatocellular carcinoma (HCC).¹⁻³ In addition to liver-related sequelae, chronic HCV infection is associated with changes in organ systems outside the liver, including metabolic, cardiovascular, and neurologic systems, and with autoimmune and immune-mediated conditions such as mixed cryoglobulinemia, thyroid disease, and glomerulonephritis.⁴⁻⁷ A large, prospective cohort study found that patients with chronic HCV infection, defined as having detectable HCV RNA in the serum, have an elevated risk of death from both hepatic and non-hepatic diseases, including cardiovascular and renal diseases, compared with uninfected patients and those with anti-HCV but no detectable HCV RNA in serum.⁸ These and other findings raise the question of whether successful treatment of chronic HCV may also improve the associated extra-hepatic effects and reduce non-hepatic morbidity and mortality. One multicenter international study has already demonstrated that achieving SVR reduces not only liver-related but also all-cause and non-liver-related mortality.⁹

The mechanisms causing the extra-hepatic effects of HCV are incompletely understood. HCV drives clonal expansion of B cells^{10, 11} to generate IgM rheumatoid factor in susceptible individuals that results in immune complex deposition in small vessels and a vasculitis, although susceptibility factors are unknown. The mechanisms of other manifestations are multifactorial, including a direct interaction between viral proteins and intracellular signaling pathways, or viral replication in extra-hepatic cells, or a heightened immune reaction with systemic effects. Immune activation may lead to a chronic inflammatory state that can affect a number of systems, as has been observed in HIV infection.¹² Like HIV, HCV infection is associated with a decreased quality of life,¹³ with fatigue, depression and cognitive impairment being the most important drivers.¹⁴ There is evidence that treatment to eradicate HCV infection may improve

some extra-hepatic manifestations of HCV independently of the severity of the underlying liver disease. The evidence is strongest for mixed cryoglobulinemia, which often resolves entirely with viral clearance.¹⁵⁻¹⁷

In the era of interferon-based treatment, extra-hepatic manifestations of HCV were frequently regarded as contraindications to treatment because the treatments could exacerbate them, or because ongoing treatment of coexisting extra-hepatic syndromes may have resulted in untoward drug–drug interactions or additional toxicities. Patients with a history of autoimmune disease or psychological instability, for example, are often ineligible for interferon-containing regimens.^{18, 19} Recent advances in anti-HCV therapy have led to well-tolerated, interferon-free regimens, such that more patients may be treated, leading to the potential for improvements in extra-hepatic manifestations on a larger scale. While previously, quality of life decreased during antiviral therapy, interferon-free therapies may improve quality of life while patients are on treatment,^{20, 21} and allow treatment where previously contra-indicated.²² This review will consider the impact of chronic HCV infection on sites outside the liver, focusing on immunological, metabolic, cardiovascular, and neurologic manifestations.

Metabolic manifestations of HCV infection

Diabetes mellitus and insulin resistance

Several studies have shown that patients with chronic HCV infection have an increased risk of diabetes mellitus compared with uninfected individuals (**Table 1**).^{5, 6, 23} White and co-workers showed that HCV infection is associated with an increased risk of diabetes in comparison to both uninfected and hepatitis B virus (HBV)-infected controls, suggesting that HCV plays a specific role in conferring the increased diabetes risk.²⁴ The elevated risk is likely due to an association between HCV and insulin resistance. Recent data suggest that HCV-induced liver

inflammation may significantly increase this risk,^{25, 26} and the observation that increased levels of liver enzymes, rather than HCV infection, is the true risk factor of diabetes development in the HCV-infected US population²⁷ should be interpreted in view of the above findings.

HCV-infected patients have significantly higher levels of insulin resistance (as measured by the homeostasis model for assessment of insulin resistance [HOMA-IR]) than uninfected controls or HBV-infected patients matched for BMI, waist circumference, age, and sex (**Table 1**).^{28, 29}

However, the evidence in favor of a viral dose-effect is weak: although patients with higher viral load tend to have higher levels of insulin resistance,²⁹⁻³¹ the correlation between HOMA-IR score and HCV RNA levels is on average very weak or absent.^{32, 33} Similarly, there is no consistently reported genotype-specificity associated with HOMA-IR levels.³⁴⁻³⁷

The most compelling evidence that HCV causes insulin resistance is the observation that curing HCV with antiviral therapy results in reduced levels of insulin resistance, while levels remain unchanged in virologic non-responders³⁸. A phase 1 study of an interferon-free, short course of danoprevir, an inhibitor of the HCV non-structural 3 (NS3) serine protease, showed a close correlation between viral load decline and reduction of HOMA-IR levels.³⁹ This suggests that treatment with HCV protease inhibitors or other anti-HCV DAAs may restore insulin sensitivity in chronic HCV-infected patients.

Mechanisms of HCV-induced insulin resistance

HCV may directly interfere with the insulin signaling pathway. This is suggested by the finding that non-obese, non-diabetic, HCV-infected individuals have hepatic insulin resistance, as determined by the hyperinsulinemic euglycemic clamp technique. Two different studies showed that endogenous glucose production in such patients was incompletely suppressed by low-dose insulin.^{34, 40} In one study, the hepatic insulin resistance index increased by a factor of 3

compared with healthy controls.³⁴ When liver samples from HCV-infected patients and uninfected controls were challenged with insulin *ex vivo*, the insulin-induced activation of the protein kinase B/Akt, the key kinase responsible for most metabolic effects of insulin, was blunted in HCV-infected cells compared with controls.⁴¹ According to experimental models, the HCV core protein seems sufficient to induce insulin resistance *via* several post-receptorial mechanisms.⁴²

In addition to hepatic insulin resistance, however, peripheral insulin resistance is also elevated in HCV infection, and appears to be the most important component of HCV-associated whole body insulin resistance^{34, 40}. An increased peripheral insulin resistance was reported independently by the above cited two groups who used a hyperinsulinemic euglycemic clamp in non-obese, normoglycemic, HCV-infected patients.^{34, 40} Using high insulin concentrations, glucose uptake and oxidative consumption were impaired, implying a deficient glucose transport and disposal, accounted for by striated muscle. Interestingly, this viral-associated insulin resistance does not appear to involve increased free fatty acid efflux from adipose tissue, which remains normally sensitive to insulin.^{34, 40} Thus, glucose uptake is clearly impaired in patients with HCV infection.

Finally, the HCV-induced liver inflammation^{25, 26} may increase the risk of developing IR *via* the release of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)- α and interleukin-6, which may in turn interfere with the insulin signaling transduction pathway in hepatocytes.⁴³

In summary, HCV causes hepatic and extra-hepatic insulin resistance. While hepatic impairment of insulin effects may be mediated by direct interactions within infected hepatocytes, increased peripheral insulin resistance may be caused by endocrine effects of soluble mediators secreted by infected hepatocytes. These soluble mediators may also increase hepatic insulin

resistance via paracrine mechanisms. They may exert their peripheral effects by reducing glucose uptake and oxidative consumption by extra-hepatic tissues, specifically muscle, and probably to a lesser extent, adipose tissue.^{34, 40} The factors involved in the paracrine and endocrine propagation of insulin resistance are currently unknown. In addition to TNF- α , other candidate cytokines include interleukin-8,^{44, 45} chemokine (C-C) motif ligand 2 (CCL2),⁴⁵ interleukin-18,³⁴ chemerin⁴⁶ and visfatin.⁴⁷

Insulin resistance and outcomes

HCV-associated insulin resistance correlates with poor outcomes, including accelerated progression of hepatic fibrosis, reduced SVR rates, and the development of HCC, and of type 2 diabetes and its cardiovascular sequelae.⁴⁸⁻⁵¹ There is no clear evidence that insulin resistance promotes HCV replication: treatment of chronic HCV with insulin sensitizers such as pioglitazone reduced insulin resistance, but failed to reduce viremia.⁵² However, among chronic HCV-infected patients with minimal or no fibrosis (F0–F1), the HOMA-IR score was independently associated with the progression of fibrosis.⁴⁸ Higher HOMA-IR scores have also been shown to be associated with lower SVR rates in patients treated with interferon, independently of HCV genotype.^{49, 50, 53-55} Finally, a large retrospective study showed that among HCV-infected patients, the presence of type 2 diabetes increases the incidence of HCC nearly two-fold compared with non-diabetic patients.⁵⁶ Controlling glycemia seems to make a difference: a higher incidence of HCC occurred in patients with HbA1c $\geq 7\%$ than among those with HbA1c $< 7\%$.

In contrast to its effect on outcomes with interferon-based treatment, insulin resistance appears to have no effect on outcomes following treatment with DAAs. A study of danoprevir monotherapy showed that the rate of HCV RNA decline was not associated with baseline HOMA-IR scores,³⁹ and two studies using telaprevir-based regimens showed that HOMA-IR

scores failed to predict virologic endpoints, including SVR.^{57, 58} However, telaprevir-based regimens still contain peginterferon and ribavirin. The availability of DAAs may enable the successful treatment of more HCV-infected patients, since even those previously classified as difficult-to-treat due to baseline insulin resistance may have improved outcomes with new therapies that do not include interferon.

Cardiovascular manifestations of HCV infection

HCV as a cardiovascular risk factor

Despite the association of HCV with insulin resistance, type 2 diabetes, and hepatic steatosis, it is still unclear whether HCV is an independent risk factor for cardiovascular disease.⁵⁹ HCV-infected patients have significantly lower levels of total cholesterol, low-density lipoprotein, and triglycerides, and higher levels of high-density lipoprotein, than uninfected individuals,^{60, 61} thus demonstrating what has been called a cardioprotective lipid profile. Nevertheless, data from several studies show an association between HCV infection and atherosclerotic changes. A case-control study showed that the prevalence of HCV positivity was significantly higher among patients with angiographically documented coronary artery disease (>50% stenosis) than among controls (patients hospitalized for other cardiac abnormalities), and that the HCV prevalence increased with the number of arteries affected.⁶² On multivariate analysis, HCV seropositivity was identified as an independent predictor of coronary artery disease, with an odds ratio of 4.2 (95% CI 1.4–13.0). Another study showed that HCV infection is an independent predictor of coronary atherosclerosis severity, with an odds ratio of 2.0 (95% CI 1.6–2.6).⁶³ Studies of changes in the carotid arteries have yielded similar results, showing an association of HCV infection with early, asymptomatic carotid atherosclerosis, as indicated by carotid intima-media thickness and the presence of plaques.^{64, 65} The HCV viral load is independently

associated with carotid atherosclerosis, suggesting a causal link between the level of HCV infection and atherosclerotic changes.³² Accordingly, a study reported that the risk of peripheral artery disease in HCV infection is higher than in uninfected patients, especially in the presence of comorbidities.⁶⁶

Consistent with the association of HCV with atherosclerosis, rates of cardiovascular events and mortality may be elevated among HCV-infected patients. A retrospective study of first-time blood donors showed increased rates of overall and cardiovascular mortality among anti-HCV-positive compared with anti-HCV-negative individuals, with a hazard ratio (HR) of 2.21 (95% CI 1.41–3.46).⁶⁷ A recent prospective study confirmed these findings, showing that HCV-infected patients have increased hepatic and extra-hepatic mortality, including increased mortality from circulatory diseases (HR 1.50; 95% CI 1.10–2.03).⁸ Further, among anti-HCV-positive individuals, the increased mortality from circulatory diseases held for patients with detectable HCV RNA, but not for those with undetectable HCV RNA, suggesting a causal connection between the virus and circulatory mortality. A recent cohort study in Taiwan found that chronic HCV infection is an independent predictor of stroke, with an adjusted HR of 1.27 (95% CI 1.14–1.41), and a community-based prospective study found that chronic HCV infection is an independent predictor of cerebrovascular death, with a significant association between cerebrovascular mortality and increasing serum HCV RNA levels.^{68, 69}

However, some studies have found no association between HCV infection and cardiovascular disease. A large retrospective study in the UK showed no difference in the incidence of acute myocardial infarction between HCV-infected and -uninfected patients,⁷⁰ and a case-control study of active-duty US military personnel similarly found no association between HCV and myocardial infarction.⁷¹ Several cross-sectional and longitudinal studies have demonstrated the lack of an independent association of HCV with atherosclerosis incidence or severity.^{72, 73} The

contradictory findings regarding HCV infection and cardiovascular disease may be due to differences in the characteristics of populations studied, or in the assessment of endpoints or adjustments for confounding factors. In addition, stratification for the prevalence of well known cardiovascular risk factors like smoking, diabetes and hypertension could help to better understand the reported conflicting results, and to identify groups of patients where the effect of HCV on cardiovascular risk is further pronounced.

Potential mechanisms of cardiovascular effects

The potential association between HCV infection and cardiovascular risk raises the question of the mechanism underlying this association. Metabolic factors may play a role: the insulin resistance associated with HCV leads to hyperglycemia, endothelial dysfunction, and inflammation, all of which produce vessel damage and unstable plaques. Perticone and co-workers recently demonstrated a significant correlation between insulin resistance and left ventricular mass among normotensive patients with HCV infection, and a strong relationship between HCV viral load and both of these parameters.⁷⁴ The presence of HCV may also induce a chronic inflammatory state with systemic effects. In support of this, Maruyama and co-workers, in a myocardial scintigraphy study of HCV-infected patients, found that 87% had myocardial perfusion defects, and that the severity of the defects was associated with the degree of liver necroinflammatory activity.⁷⁵ These findings suggest that the pro-inflammatory environment leading to necrosis and consequently fibrosis in the liver also has systemic effects, leading to atherosclerosis in the vessels. Consistent with this suggestion, Petta and co-workers found that severe hepatic fibrosis (F3/F4 vs F1/F2) was independently associated with carotid plaque development in HCV patients.⁶⁵

HCV and cardiovascular outcomes

If active HCV infection is associated with cardiovascular risk, then HCV eradication might reduce that risk and reduce cardiovascular events. Some studies have demonstrated such a connection. In the above-mentioned myocardial scintigraphy study, successful HCV RNA suppression during treatment and eradication post-treatment with interferon-based therapy was associated with improvements in the baseline myocardial perfusion defects.⁷⁵ Moreover, among patients who relapsed, there was initial improvement during HCV RNA suppression, then worsening of the perfusion defects at the time of re-appearance of the virus, while among non-responders, no change in perfusion defects was observed. Regarding cardiovascular events, a large, retrospective cohort study found that interferon-based therapy significantly reduced stroke incidence compared with no treatment (adjusted HR 0.39; 95% CI 0.16–0.95),⁷⁶ suggesting the potential long-term extra-hepatic benefits of successfully treating HCV infection. Consistent with these results a recent study from Taiwanese patients with HCV infection showed that interferon-based treatment significantly reduced the incidence of end-stage renal disease, acute coronary syndrome, and ischemic stroke.⁷⁷

Neurologic manifestations of HCV infection

Neuropsychiatric symptoms associated with HCV

Chronic HCV infection is associated with psychiatric co-morbidities. Fatigue, depression, anxiety, bipolar disorder, and schizophrenia are all more prevalent among HCV-infected patients than the general population.⁷⁸ This is partly related to the higher incidence of risk behaviors among persons with psychiatric disorders that can result in HCV exposure and increased alcohol use.⁷⁹ However, emerging literature demonstrates that HCV is also associated with an increased prevalence of neuropsychiatric symptoms, independent of pre-existing mental disorders or high risk behaviors (**Table 2**). HCV-infected patients have a

significantly reduced quality of life, as manifested by physical symptoms including fatigue, energy level, and physical functioning, compared with both uninfected and HBV-infected controls.⁸⁰ Moreover, this reduced quality of life could not be attributed to cirrhosis since patients with cirrhosis were excluded from the study, and was found to hold true for patients with or without a history of substance abuse and with either mild or severe liver necroinflammation. Thus, the symptoms causing the reduced quality of life appeared to be due to the presence of HCV, regardless of the mode of acquisition of HCV or the severity of liver disease. In subsequent larger studies, the mental aspects of health-related quality of life (HRQL) appeared specifically impaired in HCV infection compared with primary biliary cirrhosis, in which physical well-being was more impaired.⁸¹

Fatigue and cognitive impairment have both been associated with HCV infection and are in part responsible for the reduced quality of life. Fatigue is the most common symptom, reported by more than 50% of HCV-infected patients.⁸²⁻⁸⁴ Fatigue does not appear to be associated with HCV RNA level, HCV genotype, or liver histology. In one study, HCV-associated fatigue was found to be associated with female gender and age >50 years.⁸² In addition to fatigue, impaired cognition is also reported, often referred to by HCV-infected patients as a feeling of “brain fog.” Indeed, studies have demonstrated mild but significant neurocognitive impairment in a proportion of HCV-infected patients with minimal or absent liver disease.^{85, 86} In one study, in an attempt to control for factors related to modes of HCV acquisition, Forton and co-workers compared HCV-infected viremic patients with histologically mild disease to patients with prior HCV infection who had cleared the virus.⁸⁵ HCV-infected viremic patients demonstrated greater cognitive impairment on formal testing than those with resolved infection, and the deficits were shown specifically in concentration and speed of working memory. Since patients with advanced fibrosis or cirrhosis were excluded from the study, the impairments could not be accounted for

by mild hepatic encephalopathy. Moreover, the cognitive impairment was found to be independent of a history of substance abuse, depression, fatigue, or symptom severity. Thus, cognitive impairment appeared to be associated with the presence of HCV, independent of how the infection was acquired or the presence of other neuropsychiatric symptoms.

Other studies have also demonstrated HCV-associated cognitive impairment, reporting deficits in measures of immediate and sustained attention, higher executive function, verbal learning ability, recall, and working memory.⁸⁷ HCV-infected patients with mild liver disease showed deficits in attention and higher executive function compared with healthy controls, and the deficits were associated with fatigue severity.⁸⁶ Although the HCV-infected patients in that study were also more depressed and anxious than the healthy controls, the selective nature of the cognitive deficits made it unlikely that the depression caused the cognitive impairment. Hilsabeck and co-workers found a significant relationship between cognitive test performance and fibrosis stage on liver biopsy in HCV-infected patients; however, patients with minor hepatic injury also demonstrated cognitive dysfunction in the attention and concentration domains, with impairment found in up to 50% of non-cirrhotic, HCV-infected individuals.^{7, 88} The pattern of impairment found was similar to that found in other studies, and was thought to be consistent with frontal–subcortical dysfunction, which parallels findings in HIV infection. Indeed, HCV infection appears to be an important independent factor for cognitive impairment in HCV–HIV co-infected individuals.⁸⁷ For example, in a large prospective cohort of 526 patients, HIV, HCV, and methamphetamine use were independently associated with worse cognitive impairment, and higher HCV viral loads were positively correlated with impaired memory.⁸⁹

In summary, a number of studies show that patients with histologically mild hepatitis C have evidence of cognitive impairment and exhibit symptoms of fatigue, and these neuropsychiatric manifestations appear to be independent of a history of substance abuse or the presence of mood disorders. Although HCV infection is often accompanied by advanced liver disease, illicit drug use, and other factors that may have additional effects on cognitive function, the reported findings suggest that HCV infection itself may have a direct biological effect on the central nervous system (CNS).

Evidence for a biological effect on the CNS

In order to determine whether a biological process underlies the neuropsychiatric symptoms and deficits associated with HCV, brain imaging has been employed. Using proton magnetic resonance spectroscopy (MRS), several groups have shown that HCV-infected patients with mild or absent liver disease and cognitive impairment have altered cerebral metabolism.^{85, 86, 90} Cerebral proton MRS has shown that HCV-infected patients have elevated levels of choline in certain brain regions (basal ganglia, white matter, occipital grey matter) and reduced levels of N-acetylaspartate compared with uninfected individuals.^{86, 90-92} These observed changes were not associated with the severity of liver disease and could not be attributed to hepatic encephalopathy. More recently, in separate studies by Bokemeyer and Forton, HCV-infected patients were shown to have elevated myoinositol:creatinine ratios in the white matter that, in one study, were statistically correlated with impairments in working memory.^{92, 93} These findings strongly suggest that HCV infection causes brain dysfunction.

One possible mechanism by which HCV may result in brain dysfunction is by inducing neuro-inflammation. Elevated choline and myoinositol levels, demonstrated in the studies described

above, are also observed in neuro-inflammatory conditions such as multiple sclerosis or HIV infection of the brain, and are consistent with CNS glial cell proliferation and cell membrane injury, respectively.⁹⁴ The altered cerebral metabolism observed in chronic hepatitis C patients suggests that active HCV infection might result in cerebral immune activation. Further evidence for this hypothesis comes from cerebral positron emission tomography (PET) imaging using PK11195, a ligand to the peripheral benzodiazepine receptor or translocator protein, which is expressed on activated microglia. In a study of patients with histologically mild HCV infection, PK11195 binding potential was significantly increased in the caudate nucleus of HCV-infected patients compared with healthy controls and this was positively correlated with viral load.⁹⁵ The HCV-infected patients in this study also demonstrated elevated myoinositol:creatine and choline:creatine ratios compared with healthy controls. Thus, both altered cerebral metabolism and increased microglial activation were observed in patients with mild hepatitis C, and the microglial activation was associated with HCV viremia. These results may indicate that HCV in the CNS induces neuro-inflammation. Abnormalities in cerebral glucose metabolism and neurotransmission have also been reported in patients with non-cirrhotic HCV infection, suggesting that the neuro-inflammatory process leads to functional deficits.^{96, 97}

HCV may cause neuro-inflammation by penetrating the CNS and replicating in brain tissue. Evidence for this hypothesis comes from studies using molecular virology and laser capture micro-dissection techniques in autopsy samples.^{98, 99} In one study, HCV NS3 protein was found in brain microglia and, less often, in astrocytes.⁹⁸ Viral sequences isolated from the CNS are distinct from those in serum and liver and share similarities from sequences associated with or isolated from peripheral blood mononuclear cells. Another study compared autopsy brain tissue from seven HCV-positive and eight HCV-negative patients and found that microglia of HCV-positive patients expressed higher levels of pro-inflammatory cytokines than the HCV-negative controls; similarly, when microglia that co-stained for NS3 were compared with HCV-negative

cells in each of the seven HCV-positive patients, the NS3-positive cells expressed higher levels of pro-inflammatory cytokines.¹⁰⁰ These data suggest that viral penetration into the CNS may directly result in microglial activation that in turn may trigger pathways that ultimately result in disturbances in neurotransmission.

An alternative mechanism for the neuropsychiatric manifestations of HCV infection might be the effect of peripheral inflammation across the blood–brain barrier. Tryptophan, a serotonin precursor, is metabolized by the enzyme indoleamine-2-3-dioxygenase (IDO), producing kynurenine. IDO is activated by pro-inflammatory cytokines including the interferons, and its activity can be estimated by measuring the ratio of blood concentrations of kynurenine and tryptophan. Wichers and co-workers showed that in HCV-infected patients treated with interferon, the development of depressive symptoms is associated with elevated kynurenine:tryptophan ratios and the production of neurotoxic metabolites.¹⁰¹ Whether endogenous cytokines might have the same effect is not known, but in a pilot study of untreated HCV-infected patients, the kynurenine:tryptophan ratio was significantly elevated in fatigued patients compared with both HCV-infected, non-fatigued patients and uninfected controls.¹⁰²

The issue of extra-hepatic replication of HCV remains controversial, but recent work has shown that brain microvascular endothelial cells express all the receptors necessary for HCV infection and also permit HCV replication; the endothelial cells were shown to release infectious virus and to undergo conformational changes, which might allow viral passage across the blood–brain barrier.¹⁰³ One hypothesis that may explain the findings to date is that HCV may infect the brain endothelium, resulting in apoptosis and a leaky blood–brain barrier, which in turn would allow peripheral cytokine and perhaps viral entry into the CNS (**Figure 1A**).¹⁰⁴ Thus, the hypothesis is that HCV enters the brain and causes neuro-inflammation, leading to HCV-associated neuropsychiatric symptoms.

Consistent with the suggestion that there is a biological etiology for HCV-associated cognitive dysfunction, Kraus and co-workers showed that successful HCV eradication with peginterferon and ribavirin was associated with improved attention, vigilance, and working memory, while virologic non-responders showed no such improvements.¹⁰⁵ These improvements in cognitive function were demonstrated one year after the end of treatment to control for the known effect of interferon-based treatment on brain function during the treatment period.¹⁰⁶ Most recently, a pilot study in a small group of patients, using magnetic resonance spectroscopy, demonstrated normalization of cerebral N-acetyl aspartate, interpreted as recovery of neuronal dysfunction after successful antiviral treatment with interferon free treatment.¹⁰⁷

HCV, mixed cryoglobulinemia and non-Hodgkin's lymphoma

Shortly after the discovery of HCV, it was recognized that a high proportion of patients with mixed cryoglobulinemia (MC) were infected with the newly identified virus.¹⁰⁸ Subsequent studies confirmed that up to 91% of patients with MC have active HCV infection.^{109, 110} Circulating cryoglobulins are found in 40-60% of HCV-infected patients, however only 5-10% of these individuals develop clinical consequences.¹¹¹ Clinical manifestations vary widely in prevalence and severity, with many patients having no symptoms and others presenting with life-threatening systemic vasculitis. Cutaneous vasculitis with palpable purpura, often on the anterior aspect of the lower extremities, occurs in 18-33% and ranges from asymptomatic pigmentation from hemosiderosis related to past active small vessel vasculitis to aggressive cutaneous ulceration.¹⁷ Renal involvement with membranoproliferative glomerulonephritis (GN) occurs in about 27% of patients, ranging from mild proteinuria to progressive renal impairment. Other symptoms include neuropathy (11-30%), sicca syndrome (10-25%) and arthralgias (35-54%), as well as non-specific features like fatigue (50%).¹¹²

Although there is accumulating evidence that HCV is able to infect and replicate in B cells, it is not clear that lymphocyte infection is required for MC to develop.¹¹³ Clonal expansion of B cells in response to viral antigens leads to production of rheumatoid factor-containing immune complexes, which cause symptomatic disease due to a complement C1q-mediated vasculitis upon deposition in small vessels of different organs (**Figure 1B**).¹⁷ Demonstration that the cryoprecipitate contains viral antigens, particularly the core protein, along with the expected monoclonal IgM, polyclonal IgG and complement proteins, furthered the evidence supporting a direct link between HCV and MC.¹⁷ HCV may stimulate B cell proliferation through direct interaction of the HCV E2 glycoprotein with CD81 on the surface of B cells or may directly bind to and activate HCV-specific B cell receptors.¹¹⁴ B cells in patients with MC demonstrate a restricted immunoglobulin heavy chain usage, with V_H1-69 and V_K3-20 highly over-represented.¹⁰ Notably, these same clonal populations are found in patients with HCV-associated NHL, suggesting a strong link between these two lymphoproliferative conditions.¹¹ However, even among patients with MC, NHL is rare, occurring at a rate of 6.6 per 1000 person-years or lower, suggesting that NHL requires a second event beyond clonal B cell expansion.¹¹⁵ This is supported by careful evaluation of the B cell populations. Patients with MC show expansion of peripheral IgM+ κ +CD27+ B cells, characteristic of memory B cells.¹⁰ Phylogenetic analysis suggests antigen-driven affinity maturation, supporting the concept that these cells are responding to viral antigens.¹⁷ However, transcriptional analysis has shown that many of the B cells in MC patients display an anergic and pro-apoptotic phenotype, suggesting a loss of antigen-driven proliferation, possibly as a feedback mechanism to prevent auto-reactive B cell responses and explaining the relatively low frequency of clinical manifestations in patients.¹¹⁶ Loss of the pro-apoptotic phenotype through specific gene translocations, stimulation by B cell activating factor and other mechanisms may lead MC to give rise to low-grade NHL.¹¹² HCV is also associated with aggressive diffuse large B cell NHL, however the

pathogenesis may differ with less evidence of antigen-driven proliferation and a greater association with direct viral infection of B cells.^{109, 110}

The strongest support for the relationship between HCV, MC and NHL is the response to antiviral therapy. Interferon was first used to treat MC even before the discovery of HCV, with 40-60% of patients showing on-treatment responses, however with this relatively ineffective antiviral regimen, relapse was common with recurrence of MC after stopping treatment.¹⁷ With the introduction of peginterferon and ribavirin, rates of SVR increased and follow-up studies showed that 80-90% of patients had complete resolution of MC-related complications with successful viral eradication.^{17, 117} The persistence of MC after SVR may suggest that the process has become antigen-independent with continued activity due to persistent HCV antibodies despite viral clearance or possibly due to the transformation to low-grade NHL. The introduction of direct-acting antivirals holds great promise for the treatment of MC. An initial report of treatment with peginterferon, ribavirin and first-generation protease inhibitors in patients with MC found that all patients improved with therapy but notably only 70% of those who achieved SVR had a complete clinical response in terms of MC-related symptoms. Even in the 10 patients who did not achieve SVR, 60% had a complete clinical response on therapy, but 2 had a subsequent recurrence of vasculitis with viral relapse.¹¹⁸

In patients who cannot tolerate or do not respond to antiviral therapy, immunosuppressive therapy may be required. Numerous uncontrolled studies have reported beneficial effects with glucocorticoids, azathioprine and other immunosuppressive regimens.¹⁷ With the clear relationship between MC and B cell populations, it was a logical step to evaluate anti-CD20 B cell-depleting agents. Rituximab has been used alone, in combination with steroids and as an adjunct to antiviral therapy.¹⁷ The study designs and small sample sizes limit comparisons and strong conclusions but most data support that rituximab is effective in the majority of patients

and does not appear to negatively impact antiviral responses. For patients with severe, organ or life-threatening disease, plasmapheresis may be required along with B cell depleting therapy. Because of its immune-stimulatory effects, interferon may exacerbate some of the symptoms of MC vasculitis, limiting the tolerability of therapy. The introduction of interferon-free DAA regimens holds great promise for treating HCV-associated MC.

Similar to MC, even low-grade NHL may respond to antiviral therapy. The first description of regression of splenic lymphoma with villous lymphocytes with anti-HCV therapy was followed by other small series documenting regression or complete remission of HCV-associated NHL with antiviral therapy in a majority but not all patients.¹¹⁹ NHL remission has been reported in a small number of patients treated with interferon-free DAA-based regimens, suggesting that the effect is all virally-mediated and not due to anti-proliferative effects of interferon.¹²⁰ At the population level, HCV therapy has also been shown to reduce the incidence of new onset NHL in Japan, making a case for consideration of earlier treatment, even in patients with limited liver disease to prevent future complications.¹²¹ In patients with high-grade NHL, primary treatment of the malignancy is required, however once remission is achieved, antiviral therapy should be introduced, as SVR markedly reduces and may even eliminate the risk of NHL relapse.¹²² It is possible that DAA therapies could be given with or even before chemotherapy for high-grade NHL, which may improve responses and are unlikely to affect tolerability. With the remarkable progress in HCV therapy, patients with evidence of MC, even if asymptomatic, may represent a population who should be prioritized for early antiviral therapy to prevent future symptomatic vasculitis and lymphoma.

Miscellaneous manifestations

The array of extra-hepatic manifestations associated with HCV infection is large and heterogeneous. We will discuss selected ophthalmological, mucocutaneous and immunological manifestations not considered in the preceding paragraphs.

Ophthalmological

Two ophthalmological manifestations are noteworthy, i.e. Behçet disease and Mooren ulcer. In the case of Behçet disease, the causal link has never been convincingly proven,¹²³ despite initial, isolated claims.¹²⁴ On the other hand, good evidence associates Mooren-type peripheral ulcerative keratitis with HCV, since this rare condition has been reported to improve following interferon treatment.¹²⁵

Mucocutaneous

The link between HCV and lichen planus is controversial, because most studies are retrospective, making it impossible to ascertain whether HCV infection has occurred before or after the appearance of the skin lesions. In patients with oral lichen planus, HCV was shown to replicate in the oral mucosa tissue.¹²⁶ The affected oral mucosa may also harbor HCV-specific T lymphocytes,¹²⁷ underlying the pathogenetic role of HCV. However, HCV does not seem to replicate in *cutaneous* lichen planus tissue,¹²⁸ and the effect of interferon therapy in such cases has been inconsistent, thus complicating the overall picture.¹²⁹

Pruritus has been reported to occur early in the natural history of hepatitis C.¹³⁰ Pathogenesis may involve bile duct disappearance with ensuing low-grade cholestasis.¹³¹ However, in a case-control study, the prevalence of HCV was not increased among patients with pruritus, and HCV represented only a minority of the potential causal agents of chronic itching, strongly suggesting that the systematic HCV screening in such cases is not indicated.¹³²

Porphyria cutanea tarda (PCT) is the most common form of porphyria and in most cases recognizes exogenous causal agents, like iron overload, estrogen therapy, excess alcohol drinking and HCV infection.¹³³ HCV is a very frequent cause of PCT: according to a meta-analysis,¹³⁴ as many as 50% of PCT patients may have markers of HCV infection, although a wide geographical variation in prevalence suggests that other cofactors (genetic and/or environmental) may play a role in the pathogenesis and phenotypic expression of PCT. The central pathogenetic events seem to involve iron overload and oxidative stress. Although traditionally PCT has been managed by phlebotomy, the best approach is the elimination of the causal agent. Therapy with interferon and ribavirin may exacerbate PCT manifestations, including appearance of blisters in the sun-exposed areas, milia, hirsutism and skin erosions. Thus, patients with PCT may particularly benefit from interferon-free regimens.

Immunological disorders

One of the most intriguing and debated association between HCV and immunological disorders concerns rheumatoid arthritis. A recent, large population-based cohort study assessed the risk of rheumatoid arthritis in patients with a chronic infection with HCV or HBV.¹³⁵ A total of 35,652 persons had HBV infection alone, 10,253 had HCV infection, and 3,987 had chronic HBV/HCV dual infections. These were matched with 199,568 uninfected controls and followed for a decade. After adjusting for covariates, chronic HCV infection alone was significantly associated with an increased risk for rheumatoid arthritis (HR =2.03, 95% CI =1.27-3.22), a risk not shared by carriers of HBV. Convincing, conclusive data on the beneficial effect of antiviral therapy (if any) are not available.

Idiopathic pulmonary fibrosis is another rare but serious condition that has been associated with HCV infection. In a large retrospective series, the incidence of pulmonary fibrosis was significantly higher among HCV patients than in HBV infected controls.¹³⁶ Risk factors for the

development of pulmonary fibrosis were age, smoking and cirrhosis. This condition should not be banalized as it can be dramatically exacerbated by interferon therapy.¹³⁷

Thyroid autoimmune stigmata are relatively frequent in chronic hepatitis C, and may occasionally be associated with hypofunction. A genetic predisposition has been invoked, as these disorders seem to affect predominantly females with haplotype HLA DR-3.¹³⁸ Interferon alpha therapy can exacerbate this disorder; hyperthyroidism and hypothyroidism are equally observed in patients receiving interferon, with some experiencing permanent sequelae. Again, these patients may benefit from the advent of interferon-free regimens.

Finally, a troublesome association has been reported between HCV infection and some severe autoimmune cytopenias,¹³⁹ including autoimmune hemolytic anemia and autoimmune thrombocytopenic purpura. Interferon is mostly contraindicated in these patients, and, depending on the severity of the deficit, treatment requires use of corticosteroids, intravenous immunoglobulins or splenectomy. In the rare cases where interferon is permitted, the cytopenia may remit. These conditions are clearly the ultimate candidates to interferon-free regimens.

Evolving risks and benefits of HCV eradication in the era of DAAs

The availability of well-tolerated, interferon-free, DAA regimens for the treatment of chronic HCV infection will significantly broaden the spectrum of patients eligible for and willing to undergo anti-HCV treatment. Until recently, therapy may not have been indicated for patients at low risk of liver disease progression due to the numerous side effects. In particular, immune stimulation induced by interferon has been a deterrent to treat hepatitis C patients with various immunological manifestations. Further, patients with comorbid conditions such as depression, cardiovascular disease, and/or severe fatigue were typically considered poor candidates for

treatment with interferon-alpha since this drug could worsen such conditions. The recent introduction of more tolerable, more effective therapies has significantly broadened the spectrum of HCV-infected patients who can be considered candidates for treatments aimed at HCV eradication, including those who were interferon-ineligible or -intolerant, or who have a low or moderate risk of liver disease progression, or who experience mainly the extra-hepatic effects of HCV.^{22 140, 141}

Studies demonstrating that successful HCV treatment may reduce non-hepatic mortality lend credence to the concept of broader treatment indications. In a long-term study of 530 patients with advanced fibrosis or cirrhosis, SVR was associated with significantly reduced all-cause mortality.⁹ Other studies have also shown the extra-hepatic benefits of HCV eradication (**Table 3**): patients with SVR following peginterferon and ribavirin have reduced steatosis, a lower incidence of malignant lymphoma,¹²¹ reduced risk of type 2 diabetes¹⁴² and insulin resistance,^{38, 39, 143-145} improved cognitive performance,¹⁰⁵ reduction in fatigue,^{146, 147} improvement in myocardial perfusion defects,⁷⁵ reduced incidence of stroke,⁷⁶ reduced renal and cardiovascular outcomes in the presence of diabetes,⁵¹ complete resolution of MC-related complications,^{17, 117} and regression or complete remission of HCV-associated lymphoma.¹¹⁹ It is also clear that interferon and ribavirin-free treatment results in improved patient-reported outcomes in many patient groups, after as early as two weeks of treatment. Clinically important gains in quality of life are associated with SVR.^{20, 21} Thus, multiple studies have demonstrated that durable HCV eradication achieved with interferon-based therapies improves both liver-related and non-liver-related outcomes.

The availability of safe and well tolerated interferon-free regimens will enable the treatment of more patients, including those subgroups with immunological and psychiatric manifestations in whom interferon was generally contraindicated.¹⁴⁸⁻¹⁵² Indeed, clinical studies have already

assessed changes in extra-hepatic manifestations of HCV during treatment with new DAA regimens. For example, SVR achieved after treatment with sofosbuvir, including one interferon-free regimen, was associated with improvements in central fatigue¹⁵³ and in HRQL.¹⁵⁴ Data from these studies also show that patient-reported outcomes and HRQL are better during treatment with interferon-free DAA regimens than during treatment with interferon-containing regimens.^{153, 154} Thus, the evidence is mounting that viral eradication is indeed associated with an amelioration of an increasing number of extrahepatic manifestations associated with HCV, providing, apart from a clear benefit for the patient, also the support for a pathogenetic link. Not surprisingly, major clinical practice guidelines of international societies have already incorporated the presence of extrahepatic manifestations – including e.g. debilitating fatigue – as a priority indication for treatment with the novel interferon-free regimens, even in the absence of significant liver damage. However, the long-term benefit of SVR in these patients, such as the prevention of NHL in patients with cryoglobulinemia, can only be proven by large, prospective trials.

In conclusion, the involvement of non-hepatic organ systems in HCV infection substantially decreases the quality of life of chronically infected patients, and may also increase non-hepatic mortality. Viral eradication reduces extra-hepatic manifestations of HCV, and improved cure rates with new regimens will conceivably result in even more marked effects. Since these new regimens are also better tolerated than previously available treatments and have an improved risk–benefit profile, extra-hepatic manifestations of HCV form an important indication for anti-HCV treatment, even in the absence of liver disease.

Tables

Table 1. Metabolic effects of HCV infection

Potential effect	Studies and main findings	Reference
Diabetes mellitus	<i>Mehta 2000</i> : Based on NHANES data collected between 1988 and 1994, among patients 40 years and older, HCV infection was associated with diabetes (OR 3.77, 95% CI 1.80–7.87)	5
	<i>Wang 2007</i> : Compared with uninfected individuals, HCV-infected patients had a higher cumulative incidence of diabetes (HR 1.7, 95% CI 1.3–2.1) in a community-based, longitudinal study	6
	<i>Mehta 2003</i> : Among patients at high risk for diabetes, HCV infection increased the risk of diabetes more than 11-fold during 9 years of follow-up (HR 11.58, 95% CI 1.39–96.6)	23
	<i>Younossi 2013</i> : Based on NHANES data collected between 1999 and 2010, chronic HCV infection was independently associated with diabetes (OR=2.31, 95% CI 1.18–4.54), insulin resistance (OR=2.06, 95% CI 1.19–3.57), and hypertension (OR=2.06, 95% CI 1.30–3.24)	28
	<i>White 2008</i> : HCV-infected patients had significantly higher risk of diabetes compared with uninfected controls and compared with HBV-infected controls in a meta-analysis	24
Insulin resistance	<i>Younossi 2013</i> : Based on NHANES data collected between 1999 and 2010, chronic HCV was independently associated with diabetes (OR=2.31, 95% CI 1.18–4.54), insulin resistance (OR=2.06, 95% CI 1.19–3.57), and hypertension (OR=2.06, 95% CI 1.30–3.24)	28
	<i>Moucari 2008</i> : Insulin resistance (HOMA-IR) was present in 35% of HCV-infected vs 5% of HBV-infected patients, and was associated with HCV genotypes 1 and 4, high viral load, and liver fibrosis	29
	<i>Vanni 2009</i> : Patients with chronic HCV infection and no features of the metabolic syndrome (n=14) showed increased peripheral and hepatic insulin resistance compared with healthy controls (n=7); hepatic insulin resistance index was increased 3-fold in HCV-infected patients compared with controls	34
	<i>Milner 2010</i> : Insulin resistance was significantly increased in non-obese, HCV-infected male patients compared with healthy controls; insulin resistance was principally peripheral rather than hepatic, most likely in muscle	40
	<i>Muzzi 2005</i> : HOMA-IR score was associated with fibrosis in HCV-infected patients	36

	<i>Lecube 2006</i> : In a case-control study, HOMA-IR score was significantly higher in HCV-infected patients than in controls with chronic hepatitis other than HCV	155
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BMI, body-mass index; HBV, hepatitis B virus; HCV, hepatitis C virus; HOMA-IR, homeostasis model for assessment of insulin resistance; HR, hazard ratio; NHANES, national health and nutrition examination survey; OR, odds ratio

Table 2. Neurologic effects of HCV infection

Potential effect	Studies and main findings	Reference
Fatigue	<i>Foster 1998</i> : HCV-infected patients had a significantly reduced quality of life compared with uninfected and with HBV-infected controls, as measured by physical functions (fatigue, energy, body pain) assessed using the Short Form 36 (SF-36) symptomatology questionnaire	80
	<i>Poynard 2002</i> : Fatigue was present in 53% of HCV-infected patients and was associated with female gender, age >50 years, cirrhosis, depression, and purpura	82
	<i>Cacoub 2002</i> : Fatigue was present in 59% of chronic hepatitis C patients	83
	<i>Stefanova-Petrova 2007</i> : Fatigue was present in 60% of HCV-infected patients	84
	<i>Tillmann 2011</i> : Two independent prospective cross-sectional studies of 511 and 284 patients with different forms of liver disease showed reduced mental quality of life in HCV-infected patients	81
Cognitive impairment	<i>Forton 2002</i> : Chronic hepatitis C patients with detectable HCV RNA and histologically mild disease were cognitively impaired compared with previously infected patients who had cleared the virus, and compared with healthy controls; HCV-infected patients were significantly impaired on tests of concentration and speed of memory processes	85
	<i>Weissenborn 2004</i> : HCV-infected patients with mild liver disease demonstrated impairment in attention and higher executive function compared with healthy controls	86
	<i>Hilsabeck 2002 and 2003</i> : Cognitive impairment in HCV patients was related to severity of liver disease but was also evident in patients without cirrhosis	7, 88
	<i>Letendre 2005</i> : HIV, HCV, and methamphetamine use were independently associated with cognitive impairment in HCV–HIV co-infected patients	89
	<i>Weissenborn 2006</i> : Decreased serotonin and dopamine transporter binding, measured by single photon emission computerized tomography, was associated with impaired performance on psychometric testing	96
	<i>Forton 2008</i> : Impairments in working memory correlated with white matter myoinositol:creatine ratios, measured by cerebral magnetic resonance spectroscopy	93

HBV, hepatitis B virus; HCV, hepatitis C virus

Table 3. Benefits associated with eradication of HCV infection

Condition	Studies and main findings	Reference
Insulin resistance	<i>Kawaguchi 2007</i> : Chronic HCV-infected patients treated with interferon-alpha with or without ribavirin who achieved SVR had significantly reduced HOMA-IR values while virologic non-responders and relapsers showed no change in HOMA-IR	38
	<i>Milner 2014</i> : Chronic HCV-infected patients (n=8) in whom HCV was eradicated following antiviral therapy had reduced peripheral insulin resistance compared with baseline; insulin sensitivity following viral eradication was comparable to matched uninfected controls	145
	<i>Moucari 2010</i> : Decline in serum HCV RNA correlated with reduction in HOMA-IR during 14 days of monotherapy with the NS3 inhibitor danoprevir, compared with HCV RNA and HOMA-IR which remained unchanged in placebo-treated patients	39
Diabetes	<i>Arase 2009</i> : In a retrospective study, SVR following treatment with interferon or interferon plus ribavirin conferred a reduced risk (by about two thirds) of developing type 2 diabetes, even after stratification according to age, cirrhosis and prediabetes	142
Stroke	<i>Hsu 2013</i> : In a retrospective cohort study, HCV-infected patients who received interferon-based therapy had a significantly reduced risk of stroke compared with untreated patients (adjusted HR 0.39, 95% CI 0.16–0.95)	76
Myocardial perfusion defects	<i>Maruyama 2013</i> : Myocardial perfusion defects, found in the majority of HCV-infected patients, improved after interferon treatment in patients with SVR; did not change in non-responders; and temporarily improved, then returned to baseline in relapsers	75
Fatigue and HRQL	<i>Cacoub 2002</i> : Achieving SVR was associated with reduction in fatigue after adjusting for age, gender, fibrosis stage, and depression (OR 0.34, p<0.001)	83
	<i>Hassanein 2004</i> : HCV-infected patients who achieved SVR with peginterferon plus ribavirin or interferon-alpha plus ribavirin demonstrated significant improvement in HRQL, as assessed by the 36-item Short Form Health Survey (SF-36) and the Fatigue Severity Scale (FSS)	146
	<i>Rasenack 2003</i> : HCV-infected patients who achieved SVR following 48 weeks of peginterferon or interferon-alpha had significantly improved HRQL compared with those without SVR, as measured by mean SF-36 scores and mean FSS scores	147
	<i>Younossi 2014</i> : HCV genotype 2 or 3-infected patients treated with sofosbuvir and ribavirin who achieved SVR had significant improvements from baseline in HRQL, as measured by fatigue, SF-36 score, emotional well-being,	153

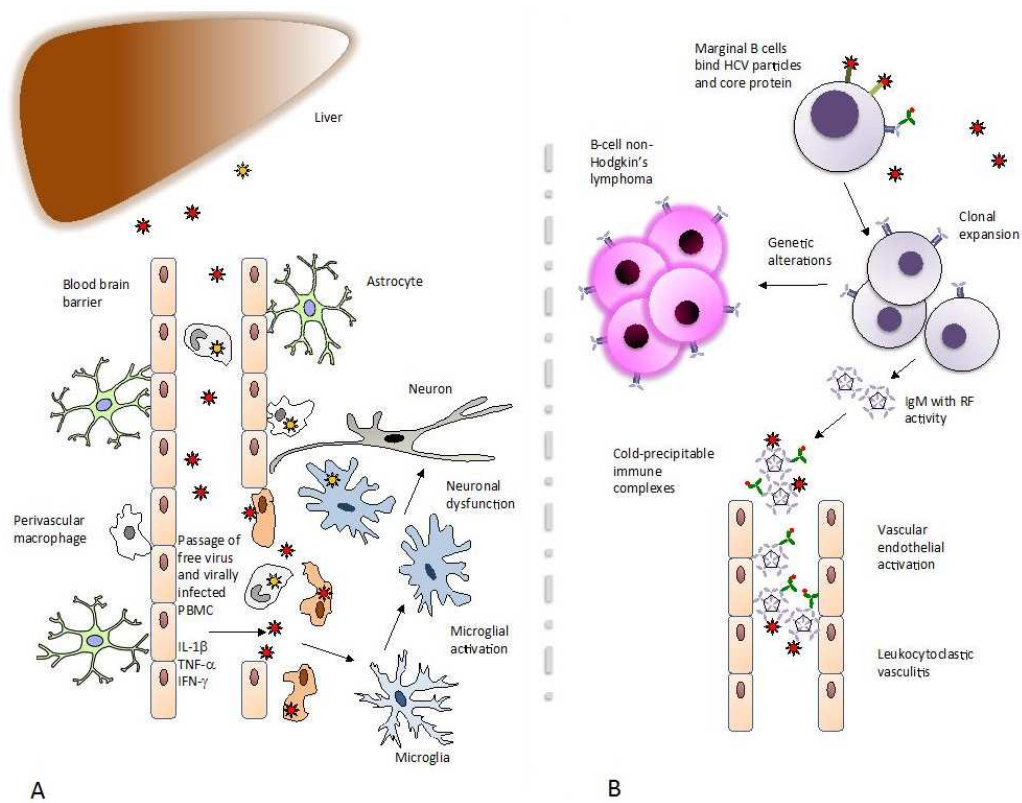
	general health, and results of the Chronic Liver Disease Questionnaire-HCV	
Cognitive function	<i>Kraus 2013</i> : HCV-infected patients with SVR after treatment with peginterferon and ribavirin demonstrated significant improvement in neurocognitive function when tested at least one year after the end of therapy; patients without SVR showed no changes in neurocognitive function	105
Cerebral magnetic resonance spectroscopy	<i>Alsop DY 2014</i> : HCV patients after treatment with ledipasvir-sofosbuvir demonstrated increases in cerebral N-acetyl aspartate, interpreted as recovery of neuronal dysfunction	107
Mixed cryoglobulinemia	<i>Gragnani L 2015</i> : HCV-infected patients with MC treated with peginterferon and ribavirin showed a good clinico-immunological correlation with SVR, since all patients with SVR also experienced a sustained clinical response, either complete or partial, while all virological nonresponders were also clinical nonresponders, despite a transient improvement in some patients	117
	<i>Saadoun D 2015</i> : 30 patients with hepatitis C and MC – mostly previous noresponders – were retreated with peginterferon and ribavirin plus a protease inhibitor (telaprevir or boceprevir), with a high rate of both clinical and virological success, in spite of side effects	118
Lymphoma	<i>Hermine O 2002</i> : In nine patients with HCV infection treated with interferon alfa, seven had a complete remission of splenic lymphoma with villous lymphocytes upon virological response, while the remaining 2 patients had a partial and a complete remission after the addition of ribavirin and disappearance of HCV RNA. One patient had a relapse when the HCV RNA load again became detectable after therapy	119
	<i>Carrier P 2015</i> : Five patients with HCV-associated B cell non Hodgkin lymphoma were treated with DAA (one received also rituximab and two chemotherapy in addition to DAA). SVR was reached in all, and complete remission of NHL was noted six months after cessation of treatment (except in one patient who had a persistent small leukemic phase)	120
	<i>Kawamura Y 2007</i> : In a retrospective study, 501 untreated and 2708 HCV-infected patients treated with interferon were followed up to 15 years. In untreated cases, a malignant lymphoma developed in 0.6% at the 5th year, 2.3% at the 10th year, and 2.6% at the 15th year. The rates in treated patients with SVR were 0% up to 15th year, while in those treated but not cured the rate increased up to 2.6% at the 15th year	121
	<i>La Mura V 2008</i> : In 69 HCV-infected patients with lymphomas, antiviral therapy carried out after chemotherapy (n=25) was associated with an increased disease-free survival.	122

	None of the SVR experienced a lymphoma relapse, while 29% of non-responders did	
Mortality	<i>Van der Meer 2012</i> : In HCV-infected patients with advanced fibrosis or cirrhosis, achieving SVR with interferon-based therapy was associated with reduced all-cause mortality	9

FSS, Fatigue Severity Scale; HCV, hepatitis C virus; HOMA-IR, homeostasis model for assessment of insulin resistance; HR, hazard ratio; HRQL, health-related quality of life; OR, odds ratio; SF-36, Short Form Health Survey; SVR, sustained virologic response

Figures

Figure 1. HCV interactions with vascular endothelium. **A.** Brain microvascular endothelial cells express all the receptors for HCV infection and are permissive to viral replication.¹⁰³ Infected endothelial cells may undergo apoptosis, inducing a conformational change, allowing a breach of the blood brain barrier. Circulating cytokines, free virus and possibly infected PBMC may passage into the CNS, leading to microglial activation and neuronal dysfunction. **B.** Cryoglobulinaemia. HCV viral particles and core protein bind to marginal zone B cells. Stimulated by B cell-activating factor, released from activated dendritic cells, there is clonal expansion of B cells leading to the release of large amounts of IgM with rheumatoid (RF) activity. IgM RF molecules form complexes with HCV viral particles to form cold-precipitable immune complexes, which accept the C1q protein and bind to vascular endothelial cells, stimulating the complement system, generating vasoactive peptides and the recruitment of neutrophils leading to a leukocytoclastic vasculitis.



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